VERSION 3

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Protocol status: Working
We use this protocol and it's working

Reverse transcription using SuperScript IV V.3

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GUIDELINES

Use high quality RNA as the substrate.

Keep RNA on ice at all times.

Use RNase free water.

MATERIALS

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SuperScript™ IV Reverse Transcriptase Thermo Fisher Scientific Catalog
#18090050

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25140

Keywords: Using
SuperScript IV to produce
cDNA from RNA

- 1 Mix the following reagents from the kit. Please scale up if more RT reaction is desired.

Reagent	Amount (uL)
Primer (Random or dT)	0.5
dNTP (10mM)	1
RNA	11

- 2 Incubate the mixture at 72 °C for 00:02:00 . Then, incubate samples on ice for few minutes.

Note

This step allows denaturation of RNA and proper priming for the downstream cDNA synthesis.

- 3 Mix the following reagents from the kit. Please scale up if more RT reaction is desired.

Reagent	Amount (uL)
RT Buffer (5x)	4
DTT(10mM)	1

RNase Inhibitor	1
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Add the 6uL to the 12.5uL mix from Step 3.

- 4 Incubate the samples at 37 °C for 00:05:00 . Then, add 1.5uL SuperScript RT IV enzyme to the reaction and mix well.

- 5 Incubate the samples using the following incubation settings:

Temp (C)	Time (minutes)
25	5
45	40
55	10
75	10

- 5.1 Add 1uL RNase H to the cDNA samples and incubate at 37 °C for 00:20:00 .

- 5.2 Dilute the cDNA samples using Nuclease free water.